C4

68. (Twice amended) The immunologically isolated marrow stromal cells of claim 37, wherein said immunologically isolated marrow stromal cells are cultured for about seven to about ten days.

REMARKS

The present invention relates to novel isolated marrow stromal cells and methods of using the cells in treatment of a variety of human diseases, disorders or conditions. The invention discloses methods comprising administering isolated marrow stromal cells into a human patient thereby effecting treatment of a disease, disorder or condition in the human.

The present invention relates to the discovery that mesenchymal precursor cells can be isolated free of hematopoietic precursor cells and used to treat a human by administering the cells. Moreover, the marrow stromal cells can be cultured *in vitro*, genetically engineered to produce therapeutic compounds, or both, prior to administration into the human.

Claims 37, 38, and 55-68 are pending in the application. Claims 1-36 and 39-44 were previously withdrawn by the Examiner as being drawn to a non-elected invention.

Claims 37, 38, and 56-58, and 65-68, have been amended to more particularly point out and distinctly claim the subject matter which Applicant regards as his invention. Support for these amendments is found in the specification as filed as more fully set forth below. Thus, no new matter has been added by way of these amendments.

Rejection of Claim 58, Under 35 U.S.C. § 112, second paragraph

Claim 58 stands rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. In the Examiner's opinion, claim 58 is vague and indefinite in that it is unclear what is meant by the term "matched donor stromal cells." More specifically, the Examiner apparently contends that although the specification as filed makes clear

that "matched donor" indicates a syngeneic donor that is matched to the recipient organism, the term is unclear in that the claim does not recite "a recipient organism."

Applicants, while not necessarily agreeing with the Examiner's reasoning, in a good faith effort to expedite prosecution of this application, have amended claim 37, from which claim 58 depends, to recite that the immunologically isolated stromal cells are implanted into the body of a recipient organism. Further, claim 58 has been amended to delete the term "matched donor marrow stromal cells" such that the claim now recites that the immunologically isolated marrow stromal cells are syngeneically matched with respect to the recipient organism.

Support for amendment of claim 37 to recite that the immunologically isolated marrow stromal cells are implanted into a recipient organism is supported throughout the specification as filed commencing on page 3, line 25. Thus, no new matter has been added by way of this amendment.

Moreover, the specification as filed, commencing on page 8, line 22, amply supports that immunologically isolated marrow stromal cells are syngeneically matched with the recipient organism in that a "matched donor" refers to "a normal, matched syngeneic donor." Further, the specification provides a specific example of a "matched donor" and a syngeneically matched recipient in that the specification discloses that matched donor cells in Example 1 were obtained from donor mice "from the same inbred FVB/N line" as the recipient mice. Thus, the disclosure in the specification as filed amply supports reciting that the cells are syngeneically matched with the recipient organism. Therefore, no new matter has been added by way of this amendment.

Applicants respectfully submit that amendment of claim 58 to recite that the immunologically isolated marrow stromal cells are syngeneically matched with the recipient organism renders the rejection under 35 U.S.C. §112, second paragraph, moot. Since claim 58, as amended, is not vague or indefinite in any way, the rejection of this claim should be reconsidered and withdrawn.

Rejection of Claims 37, 38, 55, and 57-68, Under 35 U.S.C. § 103(a)

Claims 37, 38, 55, and 57-68, stand rejected under 35 U.S.C. § 103(a) as being, in the Examiner's view, unpatentable over Carter et al. (1992, Blood 79:356-364), or, alternatively, over Cerami et al. (U.S. Pat. No. 5,846,796), taken with Ala-Kokko et al. (1991, J. Biol. Chem. 266:14175-14178), Mardon et al. (1987, Cell Tissue Res. 250:157-165), Beresford et al. (1992, J. Cell Sci. 102:341-351), and Flier (1995, Cell 80:15-18). Applicants respectfully submit that the combination of either Carter et al. or Cerami et al. with the other references (*i.e.*, Ala-Kokko et al., Mardon et al., Beresford et al., and Flier) does not render claims 37, 38, 55, and 57-68, *prima facie* obvious under 35 U.S.C. § 103(a), for the following reasons.

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

The Examiner contends that Carter et al. discloses a population of immunologically isolated stromal cells since Carter et al. teaches removing the cells from the body which, according to the Examiner, removes them from the immune system and renders the cells "immunologically isolated" as defined by Applicants in the present application.

Alternatively, the Examiner contends that Cerami et al. discloses a population of isolated mesenchymal cells comprising a gene construct operably linked to regulatory elements which function in the cells. Apparently, the Examiner continues to assert, as more fully discussed in the previous Office Action (Paper No. 14), that the

cells taught by Cerami are "immunologically isolated stromal cells" because they are removed from the body which isolates the cells from the immune system.

Even assuming, *arguendo*, that the cells taught by Cerami et al., are "immunologically isolated" as defined by the specification as filed, Applicants respectfully submit that the cells of Carter et al. and the cells of Cerami et al. are not the cells of the instant invention.

Carter et al. discloses methods of gene transfer into hematopoietic stem cells. However, the cells of Carter et al. are not the cells of Applicants' invention which are stromal cells that are mesenchymal precursor cells. *See*, *e.g.*, the specification at page 1, line 15, to page 2, line 5. That is, the specification as filed makes clear that the cells of Applicants' invention are the "adherent cells" which can differentiate into, *inter alia*, bone, cartilage, and adipocytes. These cells are distinguished from "non-adherent cells," which refers to "hematopoietic precursor cells." Specification at page 2, lines 3-5. Indeed, one of the advantages of the Applicants' invention over the prior art is the ability to separate mesenchymal precursor cells, also referred to as marrow stromal cells, from hematopoietic precursor cells. Therefore, whatever the teachings of Carter et al., may be, the reference does not teach or suggest immunologically isolated marrow stromal cells that are not hematopoietic stem cells as disclosed by Applicants.

Similarly, Cerami et al. does not teach or suggest marrow stromal cells as disclosed by Applicants. The cells of Applicants' invention are defined as follows:

> As used herein, "stromal cells", "colony forming fibroblasts", "marrow stromal cells", "adherent cells" and "MSCs" are used interchangeably and meant to refer to the small fraction of cells in bone marrow which can serve as stem-cell-like precursors of osteocytes, chondrocytes, and adipocytes and which can be isolated from bone marrow by their ability adhere to plastic dishes. (Specification at page 4, line 34, to page 5, line 6).

Therefore, the cells of Applicants' invention, as defined by Applicants and as exemplified in the specification as filed, are obtained from bone marrow and are

characterized by their adherence to plastic. *See also* specification at page 2, lines 3-5. Further, as stated previously elsewhere herein, the cells of Applicants invention do not include hematopoietic precursor cells. Indeed, one of the advantages of Applicants' invention over the prior art is that the cells of interest, *i.e.*, marrow stromal cells, are isolated separate from hematopoietic precursor cells. Further, the specification as filed discloses that administration of the marrow stromal cells can be accompanied by administration of other cells comprising hematopoietic precursor cells. *See, e.g.*, specification at page 24, lines 9-25. Therefore, the specification makes clear that the cells of Applicants' invention are not hematopoietic precursor cells unlike the cells of Cerami et al.

In further contrast to the marrow stromal cells of Applicants' invention, the cells of Cerami et al. are isolated from peripheral blood; indeed, the cells of Cerami et al. are termed "blood-borne mesenchymal cells" and display cell surface markers CD45 and CD34 which are phenotypic markers of hematopoietic stem cells (Cerami at column 5, lines 50-52; Table 1). Further, although Cerami et al. suggests that the blood-borne mesenchymal cells can be isolated from other tissues, including bone marrow, fetal liver, or embryonic yolk sac (Cerami et al., at column 4, lines 30-34), there is no data demonstrating the successful isolation of these cells from any other tissue besides peripheral blood.

More importantly, Cerami et al., at column 4, line 23, to column 5, line 17, discloses methods of isolating the blood-borne mesenchymal cells and none of the methods disclosed by Cerami et al., teach using adherence of the cells to plastic to isolate the cells. Instead, the methods disclosed relate to use of antibodies directed against various cell surface markers. Therefore, Cerami et al., does not teach or suggest use of the cells of Applicants' invention, which are defined as adherent cells obtained from bone marrow.

Additionally, there is absolutely no mention whatsoever in Cerami et al. that the blood-borne mesenchymal cells can differentiate into adipocytes, chondrocytes, and/or osteocytes, further demonstrating that the cells of Cerami et al., are not the cells of Applicants' invention. That is, Cerami et al. teaches that the cells

are fibroblast-like cells which can proliferate *in vitro*, especially in response to granulocyte-macrophage colony stimulating factor (GM-CSF) (at column 6, lines 37-38), and which can apparently migrate into wound chambers implanted into mice (column 13, line 34, to column 14, line 3). However, Cerami et al., does not teach that the blood-borne mesenchymal cells can differentiate into osteocytes, chondrocytes, and adipocytes like the cells of Applicants' invention (*see, e.g.*, specification at page 5, lines 2-4). Indeed, Cerami et al., do not even mention that the blood-borne mesenchymal cells of their invention, which are fibroblast-like, can differentiate at all. That the marrow stromal cells can differentiate into various cell types is a crucial feature of Applicants' invention which is not taught or suggest by Carter or Cerami and readily distinguishes the cells of Applicants' invention from the cells of those references.

In addition, neither Carter et al. nor Cerami et al. teaches cells that are introduced into a syngeneic matched recipient and which are physically separated from the immune system of the recipient as recited by amended claim 37. Therefore, mere removal of cells from a body does not teach the "immunologically isolated" stromal cells of Applicants' invention. That is, the "immunologically isolated" marrow stromal cells of the present invention must be transplanted into a recipient where the cells are physically separated from the recipient's immune system as recited by amended claim 37. Thus, neither Cerami et al. nor Carter et al., teaches or suggests "immunologically isolated marrow stromal cells" as disclosed by Applicants.

In sum, neither Carter et al., or Cerami et al., teach or suggest cells that are introduced into a syngeneically matched recipient or that are physically separated from the recipient's immune system. Furthermore, Cerami et al., and Carter et al., unlike the cells of Applicants' invention, do not teach or suggest that the cells are isolated from bone marrow, selected by their adherence to plastic, not hematopoietic precursors, or "can serve as stem-cell -like precursors of osteocytes, chondrocytes, and adipocytes." Specification at page 8, lines 2-3). Thus, Carter et al., and Cerami et al., do not teach or suggest the cells of Applicants' invention.

Applicants respectfully submit that combining Cerami or Carter with Ala-Kokko, Mardon, Beresford and Flier, does not correct the deficiencies of Cerami and/or Carter since none of these references, or the combination thereof, teaches or suggests the marrow stromal cells of Applicants' invention.

Ala-Kokko has nothing whatsoever to do with marrow stromal cells. Rather, the reference teaches various nucleic acid constructs expressing mammalian procollagen genes in mouse 3T3 cells. Nothing whatsoever in Ala-Kokko teaches or suggest, *inter alia*, isolated marrow stromal cells that can differentiate into adipocytes, osteocytes, and chondrocytes as defined and exemplified by the disclosure provided in the specification as filed. Thus, Ala-Kokko does not teach or suggest the cells of Applicants' invention.

Similarly, Mardon does not teach or suggest the cells of Applicants' invention. Instead, Mardon teaches placing a mixture of marrow cells in diffusion chambers that are then implanted in an animal. There was no suggestion or teaching in Mardon relating to isolation of marrow stromal cells. Indeed, Mardon (at page 164, right column) acknowledges that "[t]here are no markers for distinguishing the fibrous tissue formed by early proliferating precursors of the stromal system from the differentiated tissue arising from fibroblasts and reticular cell lines." Thus, Mardon relates to placing a complex mixture of marrow-derived cells in chambers without any attempt to select for marrow precursor cells. Further, there is nothing in Mardon to teach or suggest that the cells in the chambers differentiated, much less that the cells of Mardon are stem-cell-like precursors of osteocytes, chondrocytes and adipocytes and which can be isolated from bone marrow by their ability to adhere to plastic dishes." That is, although there may have been osteogenesis-like process within the chambers, Mardon does not teach or suggest that this was due to differentiation of marrow stromal cells. Specification at page 5, lines 2-6. Therefore, the cells of Mardon are not the cells of Applicants' invention and Mardon cannot correct the deficiencies of Carter or Cerami and/or Ala-Kokko.

Beresford does not correct the deficiencies of Carter, Cerami, Ala-Kokko or Mardon. More specifically, Beresford does not teach or suggest a marrow

stromal cell that can differentiate into an osteocyte, chondrocytes or adipocyte. Instead, Beresford only teaches that the cells isolated from bone marrow comprise cells that can differentiate into adipocytes and cells that can differentiate into osteocytes. Beresford does not teach or suggest that the cells disclosed therein are stem-cell-like-precursors that can differentiate into several cell types, including osteocytes, chondrocytes, and adipocytes. That is, although Beresford mentions the theory that "adipocytic and osteogenic cells share a common precursor in adult marrow," Beresford makes absolutely no assertion that the reference in any way relates to the discovery and isolation of such a stem-cell-like-precursor. Thus, at most, Beresford is an invitation to try to identify and isolate the cells of Applicants' invention, but Beresford cannot render Applicants' invention obvious since there is no teaching or suggestion as to how this might be accomplished and absolutely no suggestion that Beresford has accomplished it. Therefore, because Beresford does not teach or suggest isolated marrow stromal cells as defined and reduced to practice by Applicants', Beresford, neither alone nor combined with Carter or Cerami, and Ala-Kokko and Mardon, the combination of these references cannot render claims 37, 38, 55, and 57-68 *prima facie* obvious under 35 U.S.C. §103(a).

Additionally, Flier has nothing whatsoever to do with stem-cell-like-precursor cells that can differentiate into adipocytes, chondrocytes and/or osteocytes. More specifically, Flier is a review article discussing the central role of the adipocyte in energy storage and, more specifically, in obesity. The reference has absolutely nothing to do with marrow stromal cells and, therefore, does not correct the deficiencies of Carter or Cerami, alone or combined with Ala-Kokko, Beresford and/or Mardon. Since the combination of Carter or Cerami with Ala-Kokko, Beresford, Mardon and/or Flier does not teach or suggest the isolated marrow stromal cells of Applicants' invention, the combinations of these references cannot render the claims *prima facie* obvious under 35 U.S.C. §103(a).

In addition, there would have been no motivation to combine Cerami or Carter with Ala-Kokko, Mardon, Beresford and Flier to produce immunologically isolated marrow stromal cells as defined and exemplified by the disclosure provided in

the specification as filed. This is because neither Cerami nor Carter, alone nor combined with the other references, teaches or suggests "marrow stromal cells" that are stem-cell-like-precursors that can differentiate into adipocytes, chondrocytes, and osteocytes. Thus, one skilled in the art would not have been motivated to combine Carter or Cerami with Ala-Kokko, Mardon, Beresford, and Flier, since none of these references, or the combination thereof, teaches or suggests the cells of Applicants' invention. Therefore, there would have been no motivation to combine these references since the combination does not teach or suggest "marrow stromal cells," such that the combination of Cerami or Carter with Ala-Kokko, Mardon, Beresford, and Flier, cannot render the claims *prima facie* obvious under 35 U.S.C. §103(a).

In light of the foregoing arguments, it is clear that there was no reasonable expectation of success in combining the references to produce the isolated marrow stromal cells of Applicants' invention. That is, a person of ordinary skill in the art would not expect to succeed in producing stem-cell-like-precursor cells that can differentiate into adipocytes, chondrocytes, and/or osteocytes by combining Carter or Cerami with Ala-Kokko, Mardon, Beresford and Flier since these references, alone or combine, have no suggestion or teaching as to how isolate such cells.

For the reasons discussed above, the combination of Cerami or Carter with Ala-Kokko, Beresford, Flier and Mardon, cannot render claims 37, 38, 55, and 57-68 *prima facie* obvious under 35 U.S.C. § 103(a) and, therefore, the rejection should be reconsidered and withdrawn.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been either overcome or is now inapplicable, and that each of claims 37, 38, and 55-68, is in condition for allowance. Reconsideration

and allowance of each of these claims are respectfully requested at the earliest possible date.

Respectfully submitted,

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